COMMUNICATION

ChemComm
www.rsc.org/chemcomm

# Self-immolative dendrimer biodegradability by multi-enzymatic triggering†

**Roey J. Amir and Doron Shabat***

*School of Chemistry, Raymond and Beverly Sackler Faculty of Exact Sciences, Tel-Aviv University, Tel Aviv 69978, Israel. E-mail: chdoron@post.tau.ac.il; Fax: +972 (0) 3 640 9293; Tel: +972 (0) 3 640 8340*

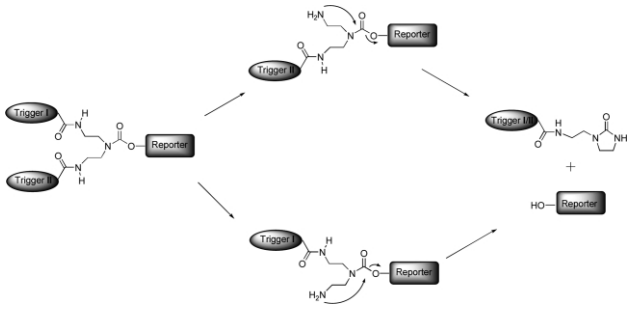
*Received (in Cambridge, UK) 5th April 2004, Accepted 15th May 2004*
*First published as an Advance Article on the web 15th June 2004*



**New self-immolative dendritic molecules have been designed and synthesized. The dendrons are built with a multi-enzymatic triggering mechanism, which initiates their biodegradation through a self-immolative chain fragmentation to release a reporter group from the focal point. The dendritic backbone is constructed from polycarbamate linkages, which are stable to hydrolysis and enhance the dendrons' solubility in water. The degradation can readily take place under physiological conditions on enzymatic triggering.**

Degradable dendrimers have been attracting special interest in the scientific community.[1,2] They are particularly desirable in the field of controlled drug delivery systems;[3–6] after a dendritic platform has ended its task as a drug carrier it needs to be cleared out from circulation. Biodegradability of a dendrimer should speed up its clearance and avoid undesired toxicity side effects.[7,8] At this time, there are only a few known examples of dendrimers that degrade by controlled fragmentation.[1,9] Recently, we and others have introduced a new class of dendritic molecules which were termed self-immolative dendrimers.[10–13] These structurally unique dendrimers can release all of their tail units, through a self-immolative chain fragmentation, which is initiated by a single cleavage at the dendrimer core.[14] We now report a new enzymatic self-immolative complete fragmentation of dendrimers, which is initiated by multi-enzymatic triggering.

A recent report appeared in literature has described dendrimers that can disassemble in organic solvents by benzyl-ether depolymerization, triggered by an allyl-ether deprotection.[15] We have extended this concept to fully biodegradable dendrimers, which have reasonable solubility in water and are disassembled through multi-enzymatic triggering followed by self-immolative chain fragmentation. The dendrimer's main building block is based on diethylenetriamine, which has two primary and one secondary amine functionalities. In a G1-dendron (Scheme 1) the secondary amine is attached to a reporter group while the two primary amines are linked to enzymatic substrates. The cleavage of either one of the substrates by the enzyme, generates a free amine group which initiates an intra-cyclization reaction to release the reporter group.




**Scheme 1** G1-dendron disassembly through a double triggering mechanism. Cleavage of either triggers **I** or **II** will initiate the release of the reporter group.

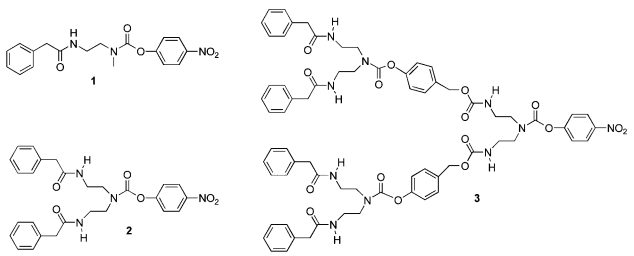
† Electronic supplementary information (ESI) available: experimental procedure and UV–visible spectra of dendrons **1** and **2**. See http://www.rsc.org/suppdata/cc/b4/b404946b/

In order to evaluate our dendrimer biodegradation pathway we synthesized G0, G1 and G2 dendrons (Scheme 2) with phenylacetamide as a triggering substrate for penicillin-G-amidase[16] (PGA) and 4-nitrophenol as a reporter group. 4-Hydroxybenzyl alcohol was employed as a self-immolative linker to connect between two amine groups through carbamate linkages.

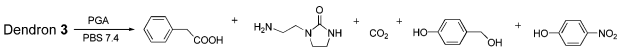
Similarly to a G1-dendron, a G2-dendron (compound **3**) can disassemble to its building blocks through the described enzymatic self-immolative fragmentation. The phenol which is released after the first intra-cyclization undergoes 1,6-quinone-methide rearrangement to release carbamic acid from the benzylic carbon. The quinone-methide species is rapidly trapped by a water molecule to yield 4-hydroxybenzyl alcohol. The generated carbamic acid undergoes spontaneous decarboxylation to form a free amine group, which self-cyclizes to release the reporter group. Importantly, only one enzymatic cleavage out of a possible four is sufficient to initiate the domino breakdown that will release the reporter group at the focal point of the dendrimer. The complete degradation of the dendron to its building blocks is depicted in Scheme 3.

The dendritic molecules were prepared straightforwardly as shown in Scheme 4 and the ESI.† Thus, dendron **1** was obtained by reaction of phenylacetyl chloride with mono-Boc-*N*-methyl-ethylenediamine to afford compound **4**, followed by Boc removal and addition of dinitrophenyl carbonate. Dendron **2** was prepared by reaction of diethylenetriamine with imidazol amide of phenylacetic acid to afford compound **5**, which was further reacted with dinitrophenyl carbonate. Coupling of compound **5** with active carbonate of 4-hydroxy-benzylalcohol afforded alcohol **6**, which was further activated with 4-nitrophenylchloroformate to give compound **7**. The latter (two equivalents) was reacted with diethylenetriamine and a subsequent one pot reaction with dinitrophenyl carbonate afforded dendron **3**.

Dendrons **1–3** were incubated with PGA in PBS pH 7.4 at 37 °C. Their biodegradation could be conveniently monitored by following the formation of 4-nitrophenol with visible spectroscopy at a wavelength of 405 nm. The kinetic release of 4-nitrophenol from the dendrons is shown in Fig. 1. Upon addition of PGA to dendrons **1–3**, free 4-nitrophenol was gradually formed, indicating that PGA cleaves its phenylacetamide substrate and the degradation indeed occurs as was predicted. As we expected the G1-dendron released




**Scheme 2** Chemical structure of G0, G1 and G2 dendrons.

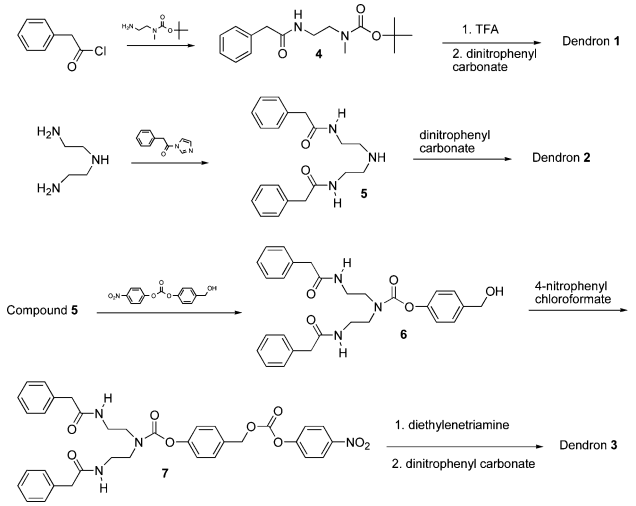



**Scheme 3** PGA catalyzed fragmentation of a G3-dendron to its building blocks.

DOI: 10.1039/b404946b

the 4-nitrophenol faster than the G0-dendron while the G2-dendron released it relatively more slowly. The background control reactions showed no release at all.

The kinetic constants $K_{obs}$ for the three reactions were calculated by linear correlation with the measured plots (Table 1). The phenomenon of dendron **2** releasing its reporter group faster than dendron **1** occurs since the enzymatic substrate concentration in dendron **2** is twice as high as in dendron **1**. The following self-cyclization step is relatively fast and therefore, the rate-limiting step is cleavage of the enzymatic substrate. In dendron **3** additional self-immolative reactions occur in order to complete the release of the reporter group (another intra-cyclization and 1,6-quinone-methide elimination). The overall rate of these reactions is slower than the rate of the enzymatic substrate cleavage and therefore the $K_{obs}$ for dendron **3** is relatively smaller.




**Scheme 4** Synthesis of dendrons **1–3**.

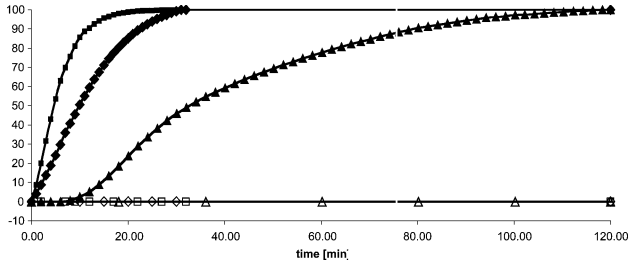



**Fig. 1** UV absorbance at 405 nm as a function of time in the biodegradation of the self-immolative dendrons. ◆ Dendron **1** + PGA. ■ Dendron **2** + PGA. ▲ Dendron **3** + PGA. □ Dendron **1** in PBS pH 7.4. ◇ Dendron **2** in PBS 7.4. △ Dendron **3** in PBS pH 7.4 (substrate concentration is 200 μM and 10 μM for PGA).

**Table 1** $K_{obs}$ values for the reporter release reactions for dendrons **1–3**

| | Dendron **1** | Dendron **2** | Dendron **3** |
|---|---|---|---|
| $K_{(obs)}$/min$^{-1}$ | 5.11 | 9.89 | 2.43 |

In conclusion, we have designed and synthesized new dendritic molecules with a multi-enzymatic triggering mechanism that initiates their biodegradation through a self-immolative chain fragmentation to release a reporter group from the focal point. For the first time, the potential of diethylenetriamine was introduced as a double trigger linker, which can be used as a building block for constructing self-immolative dendrimers. The dendrons were found to have fairly good (G0, G1) to moderate (G2) water solubility and high stability to background hydrolysis under physiological conditions. Their degradation readily occurs in aqueous medium and can easily be monitored by generation of free reporter molecule. Incorporation of different substrates on the dendron's periphery should allow the use of varying triggering enzymes.[17] This concept may be particularly important in the field of prodrug mono-therapy,[18] if a drug molecule will be incorporated instead of the reporter unit,[19,20] especially in circumstances with more than one tumor-associated or targeted enzyme with different catalytic activity. Further studies of these dendritic molecules are under progress.

## Notes and references


1 M. Smet, L.-X. Liao, W. Dehaen and D. V. McGrath, *Org. Lett.*, 2000, **2**, 511–513.
2 M. W. Grinstaff, *Chemistry*, 2002, **8**, 2839–2846.
3 Y. Kim and S. C. Zimmerman, *Curr. Opin. Chem. Biol.*, 1998, **2**, 733–742.
4 A. K. Patri, I. J. Majoros and J. R. Baker, *Curr. Opin. Chem. Biol.*, 2002, **6**, 466–471.
5 S.-E. Stiriba, H. Frey and R. Haag, *Angew. Chem., Int. Ed. Engl.*, 2002, **41**, 1329–1334.
6 D. A. Tomalia and J. M. J. Frechet, *J. Polym. Sci., Part A: Polym. Chem.*, 2002, **40**, 2719–2728.
7 H. R. Ihre, O. L. Padilla De Jesus, F. C. Szoka Jr. and J. M. Frechet, *Bioconjug. Chem.*, 2002, **13**, 443–452.
8 O. L. Padilla De Jesus, H. R. Ihre, L. Gagne, J. M. Frechet and F. C. Szoka Jr., *Bioconjug. Chem.*, 2002, **13**, 453–461.
9 D. Seebach, G. F. Herrmann, U. D. Lengweiler, B. M. Bachmann and W. Amrein, *Angew. Chem., Int. Ed. Engl.*, 1997, **35**, 2795–2797.
10 D. Shabat, *US Pat.* AN 60/406,958 2002, pending.
11 R. J. Amir, N. Pessah, M. Shamis and D. Shabat, *Angew. Chem., Int. Ed. Engl.*, 2003, **42**, 4494–4499.
12 F. M. de Groot, C. Albrecht, R. Koekkoek, P. H. Beusker and H. W. Scheeren, *Angew. Chem., Int. Ed. Engl.*, 2003, **42**, 4490–4494.
13 M. L. Szalai, R. M. Kevwitch and D. V. McGrath, *J. Am. Chem. Soc.*, 2003, **125**, 15688–15689.
14 M. Shamis, H. N. Lode and D. Shabat, *J. Am. Chem. Soc.*, 2004, **126**, 1726–1731.
15 S. Li, M. L. Szalai, R. M. Kevwitch and D. V. McGrath, *J. Am. Chem. Soc.*, 2003, **125**, 10516–10517.
16 S. P. Rannard and N. J. Davis, *Org. Lett.*, 2000, **2**, 2117–2120.
17 A. Gopin, N. Pessah, M. Shamis, C. Rader and D. Shabat, *Angew. Chem. Int. Ed. Engl.*, 2003, **42**, 327–332.
18 F. M. de Groot, E. W. Damen and H. W. Scheeren, *Curr. Med. Chem.*, 2001, **8**, 1093–1122.
19 D. Shabat, C. Rader, B. List, R. A. Lerner and C. F. Barbas III, *Proc. Natl. Acad. Sci. USA*, 1999, **96**, 6925–6930.
20 D. Shabat, H. Lode, U. Pertl, R. A. Reisfeld, C. Rader, R. A. Lerner and C. F. Barbas III, *Proc. Natl. Acad. Sci. USA*, 2001, **98**, 7528–7533.